COMPLETE DISCHARGE OF MITOCHONDRIA FROM THE SPERMATOZOON OF PERIPATUS.[1]

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The following account presents the unique case of a spermatozoön that loses all its mitochondria with the abstriction of the cytoplasm.

In my paper ('00) on the spermatogenesis of *Peripatus balfouri* the history of the germinal cycle was described from the spermatogonia through the maturation divisions, and that is now completed by a description of the spermiogenesis. In that account certain distinctive bodies were figured and discussed under the name of "yolk spherules." These were noted to occur only sparsely in the spermatogonia, but to become abundant during the growth period of the spermatocytes, and in all mitoses to lie outside of the spindles. Similar bodies were seen in the sheath cells of the testis, and I concluded these to be nurse cells, elaborators of the supposed yolk spherules, and that the spermatogonia received their "yolk spherules" from these nurse cells. At the time when that paper was sent to press Benda had not yet published his term mitochondria, and in conformity with the results of other investigators of that day I supposed true yolk to be formed within spermatocytes.

Now I am able to demonstrate that the bodies in question are not yolk spherules, rather chemically quite different from these, but are mitochondria according to their behavior and staining reactions. Indeed, it will probably be found that most bodies described as yolk spherules in spermatocytes are really mitochondria. My early account was therefore one of the first to describe mitochondria through successive cell generations of spermatogenesis. But that account was wrong in its inference that those of the germ cells proper are derived from those of the nurse cells; on the contrary, they occur independently in the

[1]*Note by the Editor.* Professor Montgomery died while this paper was in press. He had therefore no opportunity to make any changes in the proof, and the paper is printed exactly as the manuscript left his hands.

two classes of cells, just as they do in the Sertoli cells and spermatocytes of mammals.

The material consisted of testes, seminal vesicles, vasa deferentia, and oviducts, some fixed in strong Flemming's fluid diluted with an equal part of distilled water, and others preserved in corrosive sublimate-acetic; all were originally received from my friend, Dr. Purcell, of Cape Town. The mitochondria appear pale red after Hermann's safranine-gentian violet, and after the Ehrlich-Biondi-Heidenhain method; shades of gray or black after iron hæmatoxylin, according to degree of destaining; and after Benda's stain they are deep violet, while the chromatin is brownish and the centriole red—the typical reaction to this stain. There is only one other object known to me on which they are equally readily demonstrated, namely, the spermatocytes of *Ascaris*.

Fig. 1, Pl. I., exhibits the position of the mitochrondia at the end of the second maturation mitosis where, as after the first also, they lie at the distal poles (equatorial ends) of the daughter cells. Until their later fusion takes place they are chiefly peripheral, next the cell wall, spherical or slightly elongate, and in the form of hollow vesicles. In the earliest spermatids they always form a layer at the distal pole, but sooner or later move forward, along the cell membrane, so as to take a position on the side of the nucleus (Figs. 2–16); at the same time the sphere (*s*) always advances from its original position and the cytoplasm comes to make a lobe around the nucleus and entirely in front of the centriole (*c*). These movements do not occur synchronously on cells of the same stage, there is much variation in the process, yet the end result is the same in all. By reason of the mitochondria remaining generally in a single layer they may be readily counted, and their number is found to differ in different spermatids, which shows their mass cannot be accurately quartered by the maturation divisions. In Figs. 4, 6, 11–13, all of each cell are drawn with care, and their numbers here are respectively: 33, 45, 68, 64, 49. Their volumes also differ considerably, as the figures show. They do not blacken with osmic acid.

In the nucleus the chromosomes are at first peripheral and quite distinguishable (Fig. 2), then coalesce to produce a hollow

chromatin sphere enclosing clear karyolymph (Fig. 3). Next the nucleus lengthens, and distally its chromatin border becomes very thin (Figs. 4, 5, 7, 8); but it could not be determined that at this thin region nuclear sap passes out of the nucleus in the way I have described (1911) for *Euschistus*. This thin area of the nuclear wall later becomes as thick as the remainder (Figs. 9, 10). Then the proximal end of the nucleus becomes pointed (Fig. 10). With its later great elongation (Figs. 11–17, and Plate II.) the nucleus changes its appearance, due apparently to its interior becoming more chromatic, so that on surface views it appears nearly homogeneous throughout; it continues the same affinity for basic stains, and from the stage of Fig. 11 onwards I have drawn it brown and not deep black simply in order to represent the mitochondria more distinctly. Yet cross sections show that even in the mature sperm the chromatin makes a hollow cylinder and not a solid rod.

Now to return to the mitochondria, to describe their particularly notable phenomenon. After all of them have moved forward from the distal pole, carried probably by currents in the lobe of cytoplasm, they fuse together to produce a true Nebenkern or chondriosome (Meves, '00). Figs. 11–16 show them becoming agglomerated, and in Fig. 17 they are seen to be fusing to produce compound vesicles. Fig. 18, Pl. II., exhibits them on the side of the nucleus, with fusion far advanced, and Fig. 19, their consolidation into a chondriosome. As the process of fusion advances they stain more deeply, so that the chondriosomes when completed appear densely chromatic (Figs. 20–22). Sometimes a few mitochondria remain isolated without joining with the others, sometimes all fuse together (Figs. 18–21). Simultaneously the cytoplasmic lobe moves forward along the nucleus, or, probably more correctly, the nucleus moves backward through it; and in its substance appear denser strands and minute granules (Figs. 18, 19) which may be degeneration products comparable with the "tingible granules" of mammals. Consequently, each nearly mature sperm of the seminal vesicle (Figs. 20, 21), as all of the vas deferens (Fig. 22), carries near the anterior end of the nucleus a cytoplasmic lobe with a densely staining chondriosome; there appears to be no cytoplasm at all

in the region of the centriole and the flagellum. Were the history of the spermatozoa unknown beyond their conditions in the vasa deferentia, there would be no evidence of the fate of the cytoplasmic lobes and chondriosomes. But fortunately I have numerous preparations of oviducts from female individuals, all crowded with spermatozoa, and in these all the spermatozoa lack entirely the cytoplasmic lobes and chondriosomes (Figs. 23, 24); in not a single case was a cytoplasmic lobe observed upon a spermatozoön when within an oviduct.

Peripatus, accordingly, has for us more than a phylogenetic interest, it has a high cytological importance. The spermatozoön during its development casts off its cytoplasm, and evidently all of it. But this abstriction of the cytoplasm, or a portion of it, is now known to be a quite general phenomenon in animals, and only amphibians and certain insects appear to furnish exceptions to it. Much more important is that all the mitochondrial substance, in the form of a compact chondriosome, is cast away with it. Further, I had previously described the spermatozoön as possessing a lance or perforatorium, staining differently from the nucleus. Now I can demonstrate that this supposed perforatorium stains differently only on account of its excessive tenuity, that it is only the narrowed proximal end of the nucleus, and that it has no connection with the sphere. We have seen that the sphere arises just behind the nucleus (Figs. 2, 3), and moves forward into the cytoplasmic lobe (Figs. 5, 7, 8, 10, 12, 14, 16–19). When the chondriosome is fully developed the sphere lies still in the cytoplasmic lobe, separated from the nucleus (Fig. 20), and no evidence was observed that it moves along the latter to constitute a perforatorium. Therefore it is certain that the sphere as well as the chondriosome becomes thrown off with the cytoplasm.

The history of the centriole was not followed in detail. In the telophase of the secondary spermatocytes a minute centriole is present at each pole (Fig. 1). At the next stage when it was noticed (Fig. 3) it appeared as a much more voluminous body at the distal pole of the nucleus, and it retains this position thereafter. Later it becomes discoidal with indication of subdivision into two parts (Figs. 4–10), and afterwards lengthened in the axis

of the spermatid (Figs. 11–19), reaching its maximum size at the stage of Figs. 13, 14. In the mature and nearly mature spermatozoa it makes a slender rod, joining nucleus and flagellum, and then is seen to have decreased in volume (Figs. 21–24). The flagellum connected with it is a delicate, flattened thread, evidently without spiral membrane or cytoplasmic sheath; in the figures only its proximal portion is shown. In some cases there appeared to be a spiral skeleton around the nucleus, such as Koltzoff (1908) has recently described in other species; but examination proved that in *Peripatus* this is occasioned simply by chance wrapping of a flagellum around the nucleus.

In the mature egg and in cleavage stages no structures were found in any way resembling the mitochondria of the sperm cells.

Meves (1908, and later papers) draws the conclusion that mitochondria are important hereditable elements, directing cytoplasmic activities as the chromosomes direct those of the nucleus, self-perpetuating bodies differentiating during ontogeny into most of the fibrillar structures of the body. Without entering into the rapidly growing literature now, we will be content with the statement that a considerable number of investigators corroborate these views, and that they have been especially elaborated by Giglio-Tos and Granata ('08). This hypothesis more than any other has directed attention to these bodies. Yet they are far less conservative and regular than the chromosomes in number, form and behavior and there is evidence that occasionally some of them are eliminated during spermiogenesis. Thus Fauré-Fremiet has distinguished four types of them: (1) Those that undergo changes of position without profound morphological changes, as in mammalian spermiogenesis. (2) Those that at the same time undergo great structural changes, as in insect spermatids. (3) Those of which only a part change into the chondriosome or Nebenkern of the spermatid, while others degenerate, as in spermatogenesis of certain gastropods. And (4) those that transform wholly or partly into deutoplasmic bodies, some cases of oögenesis. The fourth of these classes cannot be said to be definitely established, but there can be little doubt about the evidence for the third. Thus, besides the instance in gastropod spermatogenesis studied by Fauré-

Fremiet, Retzius noted in mammals a "considerable reduction in their substance as they enter into the formation of the spiral thread." Jordan ('11) found that in spermatids of the opossum a considerable number of mitochondria are cast off with the cytoplasm; and while Duesberg ('10) maintains that in the guinea pig all take part in forming the spiral thread of the spermatozoön, yet he figures granules with similar staining reactions in the dehiscent cytoplasmic lobe. The parallel does not seem yet to have been made, yet may not the "tingible corpuscles" of mammalian spermatids be metamorphosed mitochondria, ones that have nothing to do with the spiral threads? And now we are able to adduce the positive case of *Peripatus*, in which all the mitochondria become removed from the spermatozoön.

In view of these facts it seems to me we should be very cautious in attributing to the mitochondria a rôle in cellular activity at all equal to that of the chromosomes. No spermatozoön ever discards chromosomes, but that of *Peripatus* throws off all its mitochondria.

LITERATURE CITED.

Duesberg.

'10 Nouvelles recherches sur l'appareil mitochondrial des cellules séminales. Arch. Zellf., 6.

Fauré-Fremiet.

'10 Étude sur les mitochondries des Protozoaires et des cellules sexuelles Arch. Anat. Micr., 11.

Giglio-Tos e Granata.

'08 J mitocondri nelle cellule seminali maschili di *Pamphagus marmoratus* (Burm.). Biologica, 2.

Jordan.

'11 The Spermatogenesis of the Opossum, etc. Arch. Zellf., 7.

Meves.

'00 Ueber den von v. la Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen. Arch. mikr. Anat., 56.

'08 Die Chondriosomen als Träger erblicher Anlagen. Ibid., 72.

Montgomery.

'00 The Spermatogenesis of *Peripatus balfouri* up to the Formation of the Spermatid. Zool. Jahrb., 14.

Montgomery.

'11 The Spermatogenesis of an Hemipteron, *Euschistus*. Journ. Morph. 22.

Explanation of Plates.

All figures were drawn with the cameral lucida at the level of the base of the microscope, Figs. 13, 20 with Zeiss apochr. 1.5 mm., oc. XII., the others with Zeiss achr. 1/12 mm., oc. XII. Figs. 1–3, 5, 7–12, 14–19 from a seminal vesicle, Flemming's fluid, iron hæmatoxylin; Figs. 4, 6, 21 from a seminal vesicle, corrosive sublimate-acetic, iron hæmatoxylin; Figs. 13, 20 from a seminal vesicle, Flemming's fluid, Benda's stain; Fig. 22, from a vas deferens, Flemming's fluid, safranine-gentian violet; Fig. 23, from an oviduct, treated like the last; Fig. 24, from an oviduct, corrosive sublimate-acetic, iron hæmatoxylin.

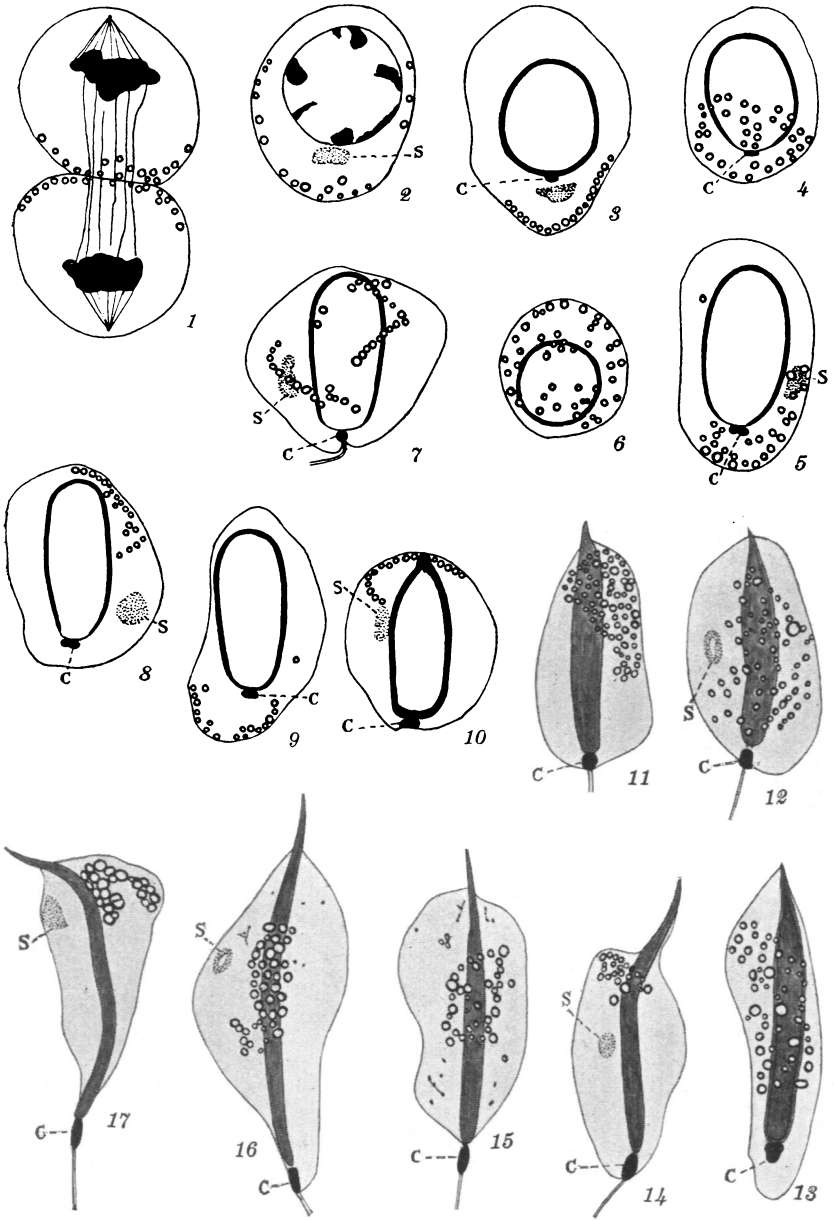
c, centriole.

s, sphere.

Plate I.

Fig. 1. Telophase of second maturation mitosis.

Figs. 2–17. Successive stages of spermiogenesis from the seminal vesicle. Fig. 6 is an apical view of the stage of Fig. 5.



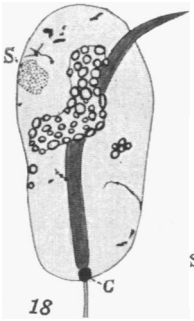
T. H. MONTGOMERY.

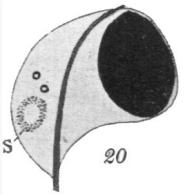
PLATE II.

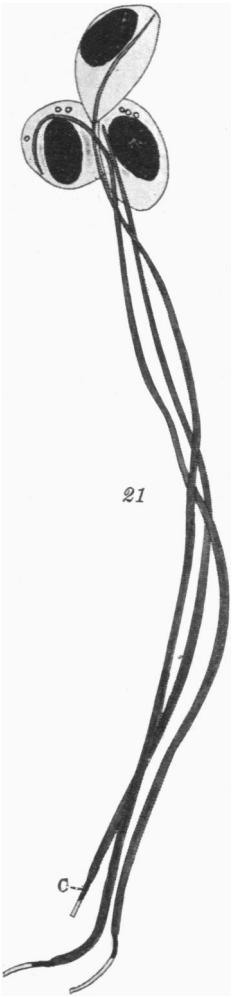
FIGS. 18–21. Later stages of spermiogenesis from seminal vesicle. In Fig. 20 only the cytoplasmic lobe and the proximal portion of the head are shown.

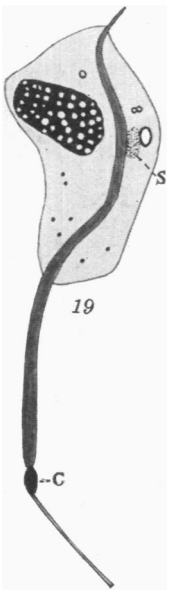
FIG. 22. Spermatozoön from vas deferens.

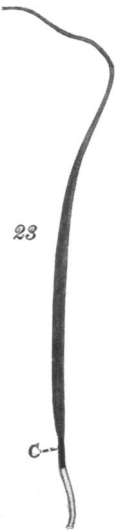
FIGS. 23, 24. Spermatozoa from oviduct.











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